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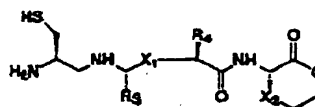
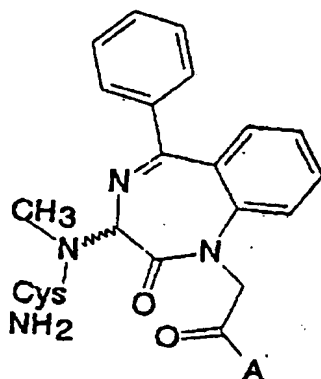
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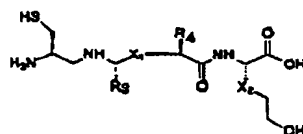
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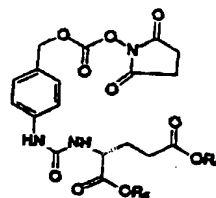
(54) Title: IMPROVEMENTS RELATING TO PRODRUGS



(Ib)



(Ic)



(57) Abstract

The present invention provides a prodrug of the general formula FTLi-(PRT)_m, where FTLi is a ras inhibitor such as a farnesyltransferase inhibitor compound and PRT represents m protecting groups capable of being cleaved from the ras inhibitor by the action of an enzyme, where m is an integer from 1 to 5.

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IMPROVEMENTS RELATING TO PRODRUGS

The present invention relates to prodrugs and their use in the treatment of tumours.

The use of prodrugs represents a clinically very valuable concept in cancer therapy since, particularly where the prodrug is to be converted to an anti-tumour agent under the influence of an enzyme that is linkable to a monoclonal antibody that will bind to a tumour associated antigen, the combination of such a prodrug with such an enzyme monoclonal/antibody conjugate represents a very powerful clinical agent. This approach to cancer therapy, often referred to as "antibody directed enzyme/prodrug therapy" (ADEPT) is disclosed in WO88/07378.

More recently, a similar approach ("VDEPT") has been proposed where in place of an antibody/enzyme conjugate, tumour cells are targeted with a viral vector carrying a gene encoding an enzyme capable of activating a prodrug. The gene may be transcriptionally regulated by tumour specific promoter or enhancer sequences. The viral vector enters tumour cells and expresses the enzyme, in order that a prodrug is converted to an active drug only in the vicinity of the tumour cells (Huber et al, Proc. Natl. Acad. Sci. USA (1991) 88, 8039). Alternatively, non-viral methods for the delivery of genes have been used. Such methods include calcium phosphate co-precipitation, microinjection, liposomes, direct DNA uptake, and receptor-mediated DNA transfer. These are reviewed in Morgan & French (Anderson, Annu. Rev. Biochem., 1993, 62;191). The term "GDEPT" (gene-directed enzyme prodrug therapy) is used to include both viral and non-viral delivery systems. The enzyme may be targeted to particular subcellular locations or expressed at the cell surface.

Although the GDEPT and ADEPT systems enhance the concentrations of anti-tumour agents which may be delivered to the site of a tumour, there is still a need to enhance the specificity of drug

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delivery. In both systems, active drug can be released into the environment of normal cells and cause damage. In the case of ADEPT, this can be caused by activation of prodrug by conjugates which have failed to localise at the tumour site. In GDEPT, transformation of normal tissue may lead to residual levels of expression of the enzyme away from the tumour or active drug may be released from tumour cells. Although ways to increase the specificity of the ADEPT system is disclosed in WO89/10140, there remains a continuing need to improve the level of ADEPT and GDEPT specificity.

Ras is often associated with transformation of cells. Oncogenic ras is over-expressed in a number of human tumours eg. over 50% of colon carcinoma and 90% of pancreatic carcinomas (Marsters et al, 1993, Science 260 1937-1942) also in lung tumours.

15 It has been shown that inhibition of oncogenic ras in cell culture leads to reversal of the transformed phenotype. Therefore the ability to develop specific inhibitors of ras may have great therapeutic application.

20 However, one potential pitfall with targeting ras, is the presence of normal non-mutated ras in all normal cells. In normal cells the role of ras is crucial. It is therefore essential to inhibit selectively only activated ras in transformed cells.

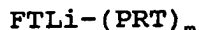
25 Recently, inhibitors of ras farnesyltransferase, which selectively inhibit farnesylation of oncogenic ras, have been described. (Kohl, Science, 260, 1934, 1993; James, ibid, 1937).

The present invention addresses such problems by the use of a novel class of prodrugs, which are prodrugs of ras inhibitors such as inhibitors of farnesyltransferase. This enzyme is essential for farnesylation of ras which is an absolute requirement for the transforming function of oncogenic ras (Gibbs, 1992, seminars in Cancer biol. 3, 383).

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The use of ras inhibitors such as farnesyltransferase inhibitors in ADEPT or GDEPT thus provides an increased level of specificity for the treatment of tumour cells. Prodrugs based upon farnesyltransferase inhibitors will be converted into farnesyltransferase inhibitors primarily at the site of a tumour, but at the same time release of farnesyltransferase inhibitors at other sites or from the tumour will not cause cytotoxicity comparable to the release of non-specific cytotoxic drugs.

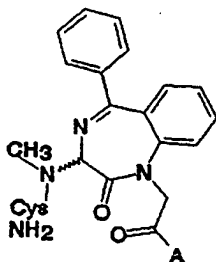
- 10 In one aspect, the present invention provides a prodrug of the general formula:



where FTLi is a ras inhibitor such as a farnesyltransferase inhibitor compound and PRT represents m protecting groups capable of being cleaved from the ras inhibitor by the action of an enzyme, where m is an integer from 1 to 5.

Suitable FTLi's include those described by James et al (ibid) and Kohl et al (ibid), and especially FTLi's of the general formula (Ia)

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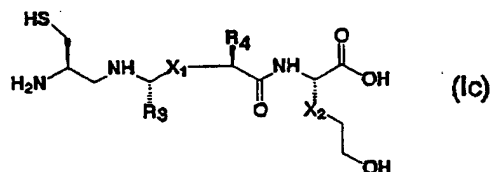
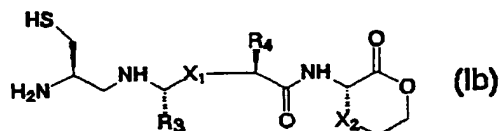
wherein Cys is cysteine; A is a group $-\text{NH}.\text{CH}(\text{R}^1).\text{COOR}^2$ wherein R^1 is a naturally occurring amino acid side chain and R^2 is hydrogen, methyl or amino. Compounds of the formula Ia in which A is a leucine $[-\text{NH}.\text{CH}(\text{CH}_2\text{CH}(\text{CH}_3)_2).\text{COOH}]$ group, a serine $[-\text{NH}.\text{CH}(\text{CH}_2\text{OH}).\text{COOH}]$ group, a methionine $[-\text{NH}.\text{CH}(\text{CH}_2\text{CH}_2\text{SCH}_3).\text{COOH}]$

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group, or an amidated methionine $[-NH.CH(CH_2CH_2SCH_3).CONH_2]$ group are preferred. Compounds of formula Ia may be used as racemic mixtures or the isomers may be isolated.

Other suitable FLTi's include those of the formula (Ib) or (Ic):



5 R_3 and R_4 are

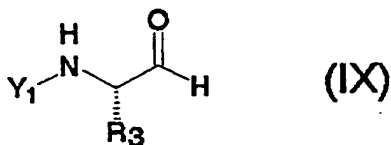
the side chains of naturally occurring amino acids (for example $-CH_3$, $-CH(CH_3)_2$, $-CH_2CH(CH_3)_2$, $-CH(CH_3)CH_2CH_3$, $-CH_2OH$, $-CH_2CH_2SCH_3$, $-CH(OH)CH_3$), including their oxidized forms (for example, methionine sulfoxide or methionine sulfone), or are substituted or unsubstituted aliphatic, aromatic or heteroaromatic groups, preferably cyclohexyl, phenyl, pyridyl, imidazolyl or saturated or unsaturated branched or straight chains of 2 to 8 carbon atoms optionally substituted with an aromatic or heteroaromatic ring;

10 X_1 is CH_2CH_2 , trans $CH=CH$ or CH_2NH ; and

X_2 is $(CH_2)_n$ wherein n is 0, 1 or 2. Preferably R_3 and R_4 both represent $CH(CH_3)CH_2CH_3$.

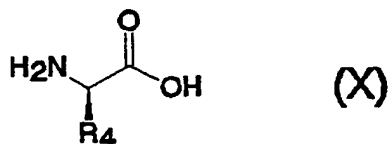
Compounds of formula (Ib) or (Ic) wherein X_1 is $-CH_2NH-$ can be obtained by the following general process. A compound of the

20 formula (IX):

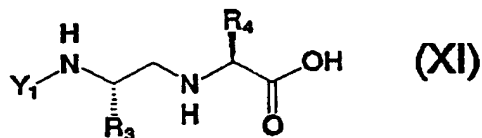


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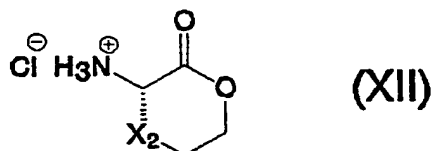
wherein Y_1 is a suitable protecting group, for example t-butoxycarbonyl, and wherein R_3 is defined as above, which may be prepared from the corresponding protected amino acid by formation of the Weinreb amide followed by reduction, preferably by $LiAlH_4$,
 5 in ether below $-35^\circ C$, is reacted with the trifluoroacetate salt of a compound of formula (X):



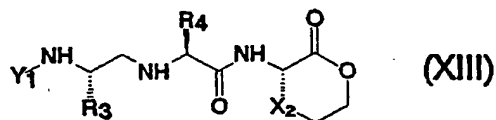
wherein R_4 is as defined above, in anhydrous DMF, followed by reduction, preferably with sodium triacetoxy borohydride to obtain a compound of formula (XI):



10 wherein Y_1 , R_3 and R_4 are as defined above. The compound of formula (XI) is then reacted in the presence of triethylamine with a compound of formula (XII):



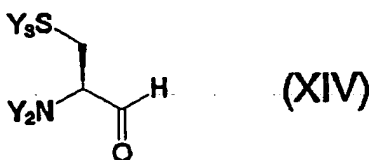
wherein X_2 is as defined above to obtain a compound of formula (XIII):



15 wherein Y_1 , R_3 , R_4 , and X_2 are as defined above. This product is then deprotected, for example, by treatment with a solution of

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anhydrous hydrogen chloride in ethyl acetate at -25°C and then reacted with a compound of formula (XIV):



wherein Y_2 and Y_3 are suitable protecting groups, for example, Y_2 is t-butoxycarbonyl and Y_3 is trityl, (compounds of formula (XIV) may be obtained from the corresponding protected cysteine by formation of the Weinreb amide followed by reduction, preferably by LiAlH_4 in ether at below -35°C), to obtain after deprotection a compound of formula (Ib), which may then optionally be hydrolysed to obtain a compound of formula (Ic).

10 The above compounds of formula (Ib) or (Ic) wherein X is $-\text{CH}_2\text{NH}-$ may also be obtained by reference to Kohl et al and James et al.

Compounds of formula (Ib) or (Ic) wherein X is CH_2CH_2 or trans $\text{CH}=\text{CH}$ may be obtained by the process described in WO 94/09766.

FTLi's may be linked to any suitable protecting group which is removable by an enzyme. Examples of such groups include those found in WO88/07378 or in WO93/08288. For example, WO93/08288 describes "self immolative" prodrugs which can be activated by the action of a nitroreductase enzyme. These prodrugs are derivatives of p-nitrobenzyloxycarbonyl compounds.

20 The exact structure of the protecting group will depend upon the nature of the ADEPT or GDEPT system with which a ras inhibitor prodrug is to be used. It may be any suitable group which can be removed by an enzyme or modified by the enzyme in such a manner that the group is unstable and undergoes "self immolation" to provide the active ras inhibitor drug.

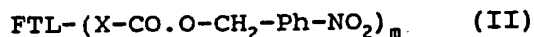
The number of protecting groups attached to each FTLi will depend in part upon the exact structure of the inhibitor compound. It

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will also depend upon the relative activity of the unprotected FTLi to the FTLi when different numbers of protecting groups are added, since if additional protecting groups will achieve a reduction in potency of the prodrug this will increase the ratio of activity of FTLi to FTLi-(PRT)_m.

Desirably, one or two protecting groups will be attached to each FTLi molecule to provide a compound of the invention, although more, e.g. 3, 4 or 5 groups may be added where the FTLi is of a structure which will allow this number to be linked.

10 Accordingly, prodrugs according to the invention include compounds with a protecting group of the formula (II):



where X is NH, O or S, m is an integer from 1 to 5 (e.g. 1, 2 or 3), Ph is an optionally substituted phenylene ring and FTL is a group such that FTL-(XH)_m is a FTLi containing m -XH groups. The
15 nitro group may be in the 2-position although is desirably in the 4-position of the ring relative to the Ph ring.

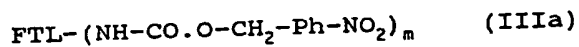
Within each compound of formula (II) where m from 2 to 5, each group X and Ph may be the same or different. Preferably, they
20 are the same.

FTL inhibitors in formula (II) include those of formula (Ia), (Ib), or (Ic) above.

Suitable substituents of the phenylene ring include 1 to 4 groups which may be the same or different which are selected from
25 fluorine, chlorine, bromine, iodine, hydroxy, mercapto, amino, nitro, C₁₋₄ alkyl, C₁₋₄ haloalkyl, C₁₋₄ alkoxy, C₁₋₄ haloalkoxy, C₂₋₄ alkenyl, C₂₋₄ alkynyl, and C₂₋₄ haloalkenyl. Preferably, the substituents are from 1 to 4 fluorines in the 2, 3, 5 and 6 positions of the ring.

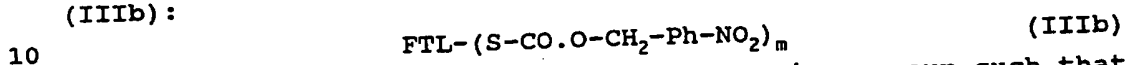
30 Preferred prodrugs according to the invention are those of formula (IIIa):

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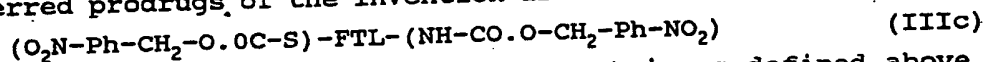
where m and Ph is as defined above and FTL is a group such that FTL-(NH₂)_m is a FTLi compound containing m amino groups. Such prodrugs include those of formula (Ib) or (Ic) above which
5 comprise at least one amino group. The nitro group may be in the 2-position although is desirably in the 4-position of the ring relative to the Ph ring.

Further preferred prodrugs of the invention are those of formula (IIIb):



where m and Ph is as defined above and FTL is a group such that FTL-(SH)_m is a FTLi compound containing m mercapto groups. Such prodrugs include those of formula (Ib) or (Ic) above which
15 comprise at least one mercapto group. The nitro group may be in the 2-position although is desirably in the 4-position of the ring relative to the Ph ring.

Preferred prodrugs of the invention are those of formula (IIIc):



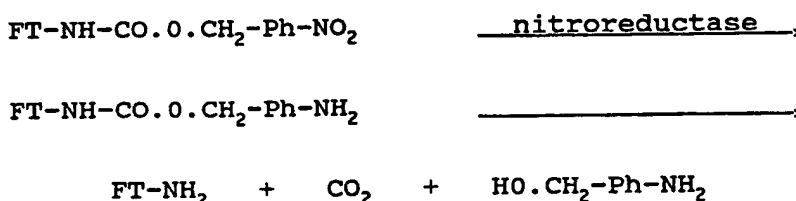
wherein Ph which is the same or different is as defined above.
20 FTL is a group such that (HS)-FTL-(NH₂) is a FTLi compound. Such prodrugs include those of formula (Ib) or (Ic) above. The nitro group may be in the 2-position although is desirably in the 4-position of the ring relative to the Ph ring.

25 Compounds of formulae (II) and (IIIa, b & c) may be used as prodrugs in an ADEPT or GDEPT system in conjunction with a nitroreductase enzyme, including the E.coli nitroreductase described in WO93/08288. While the present invention is not dependent, for its definition, upon the exact mode of action of
30 the nitroreductase on the compound of formula II or (IIIa, b or c), it is believed that the nitro group of the p-nitrophenyl-benzyloxy-carbonyl residue is converted to the corresponding amino or hydroxylamino group and that the resulting p-aminobenzyloxy-carbonyl or p-hydroxyl-aminobenzyloxycarbonyl
35 compound automatically degrades under the reaction conditions

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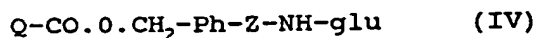
used for the enzymatic reduction to release the cytotoxic compound and form p-aminobenzyl alcohol or p-hydroxylaminobenzyl alcohol and carbon dioxide as by products in accordance with the following reaction scheme:

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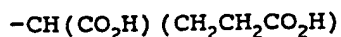


- 10 The p-nitrobenzyloxycarbonyl compounds of the invention are conveniently prepared by methods of chemical synthesis known per se. For example, suitably protected amine or hydroxy ras inhibitor compounds can be reacted with 4-nitrobenzyl chloroformate under anhydrous conditions in the presence of a
- 15 hydrogen chloride acceptor, particularly an alkylamine such as triethylamine. This reaction can be carried out in a dry organic solvent such as chloroform and the resulting compound of the invention of formula II or formula IIIa, b or c isolated from the organic solvent by conventional methods such as chromatography.
- 20 For use in ADEPT the prodrug should be unable to or have limited ability to enter cells, whereas for GDEPT the prodrug must enter cells. Accordingly, modifications may be made in the prodrug, eg in the benzene ring, to make the prodrug more, or less, lipophilic. The gamma acid of the glutamic acid may be altered
- 25 to make compounds more lipophilic, eg with an ester group.

Similar prodrugs which can be activated by a carboxypeptidase enzyme such as carboxypeptidase G2 (CPG2) can be made using derivatives of the formula (IV):

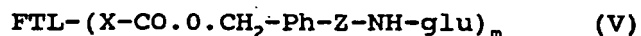


- 30 where Q is hydrogen or fluoro, chloro or bromo or -O-(N-succinimide), Ph is as defined above, Z is -O.CO- or -NH.CO- and glu is the residue of glutamic acid, ie a group:

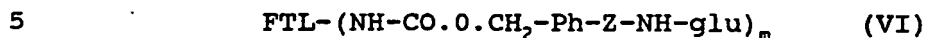


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or a di-C₁₋₆ alkyl ester (e.g. an ethyl or t-butyl ester) thereof, in order to provide prodrugs of the formula (V):



wherein X is as defined above and of the formula (VI)



where FTL is the residue of a FTLi compound such that FTL-(XH)_m and FTL-(NH₂)_m are as defined above, and where m, Ph, Z and glu are also as defined above. As mentioned above in connection with prodrugs of formula (II) and formula (IIIa, b or c), for ADEPT
10 the prodrug should have limited ability to enter cells whereas for GDEPT the prodrug may be modified if need be to make it more lipophilic in order that it does enter cells. The gamma carboxylic group of the glutamic acid may be altered to make compounds that are more lipophilic, e.g. with an aromatic or
15 heterocyclic amide.

Within each compound of formula (V) where m from 2 to 5, each group X and Ph may be the same or different. Preferably, they are the same.

In compounds of formulae (IV), (V) and (VI), the group -Z- is in
20 the 4-position of the ring relative to the FTL containing substituent.

Compounds of the formula (V) and (VI) in which the FTL is of formula (Ib) or (Ic) are preferred.

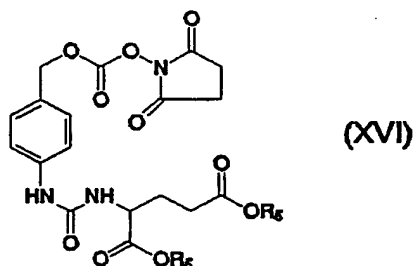
The benzyl chloroformate derivatives of the formula (IV) in which
25 Z is -NH.CO- may be made from 4-(chloromethyl)phenyl isocyanate by reaction of glutamic acid or a protected derivative thereof, eg in which both carboxy groups of the glutamic acid residue are protected with ethyl or t-butyl groups. Suitably, the reaction is carried out in a solvent such as CH₂Cl₂ at about room
30 temperature. The resulting intermediate, [4-chloromethyl]phenyl-uridoglutamate-di-tert-butylester, is treated in aqueous ethanol under reflux to provide the corresponding 4-hydroxymethyl compound and this is reacted with triphosgene ((CCl₃O)₂CO) in an

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inert solvent, eg. THF, at room temperature to provide an optionally protected compound of formula (IV). The compound when protected may be deprotected by treatment with trifluoroacetic acid or formic acid.

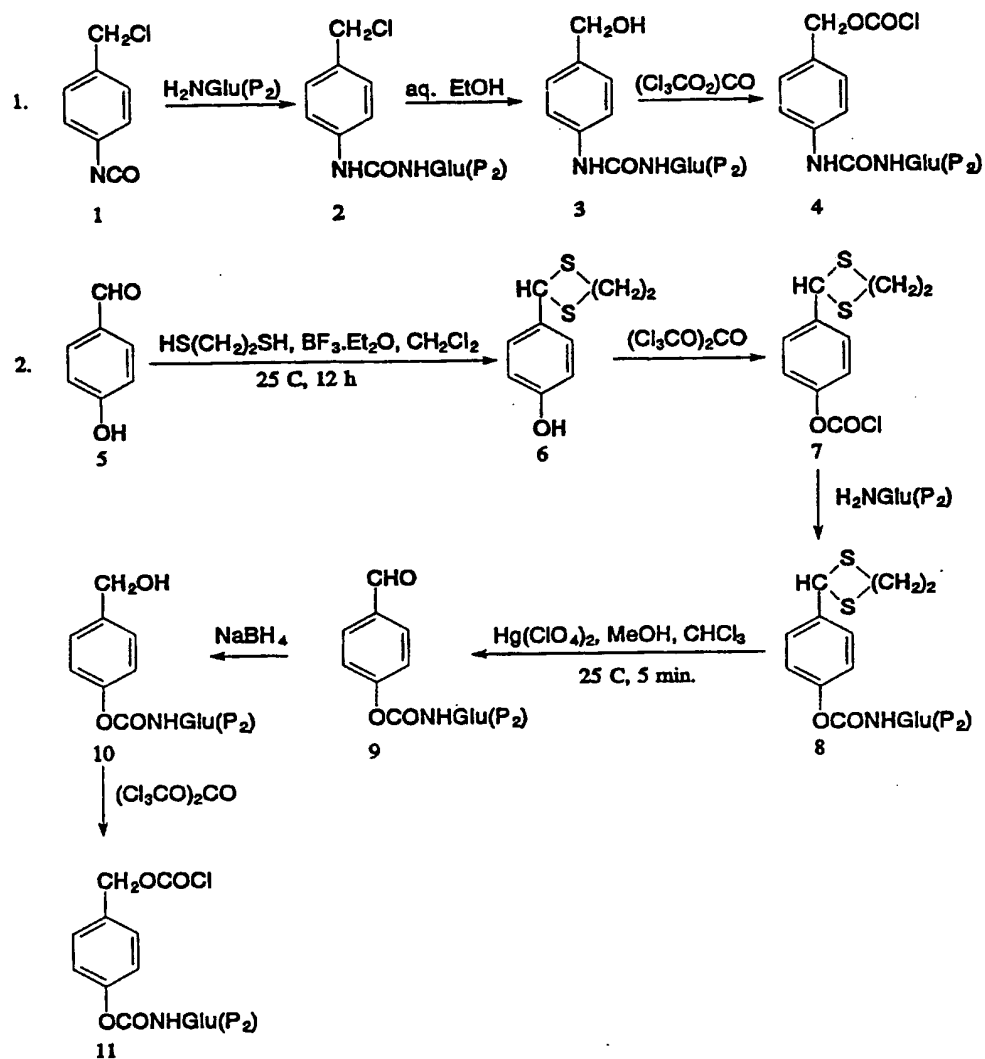
- 5 The benzyl chloroformate derivatives of the formula (IV) in which Z is -O.CO- may be made starting from 4-hydroxybenzaldehyde in accordance with the reaction scheme in Scheme 1. Briefly, the aldehyde 5 is treated with 1,2-ethane dithiol in borane trifluoroetherate plus CH_2Cl_2 at 25°C for about 12 hours to form
 10 the 1,3 dithiolane intermediate which is treated with triphosgene as above to form the 4[1,3 dithiolane] phenylchloroformate. This is coupled with di-tert-butyl-glutamate hydrochloride in dry THF in the presence of triethylamine at room temperature for about 5 hours, to provide 4[1,3 dithiolane] phenylcarbamate-glutamate-
 15 di-t-butyl. The dithiolane is deprotected with mercuric perchloroate in methanol and chloroform at about 25°C for about 5 minutes. The aldehyde is converted to the corresponding benzylic alcohol by mild reduction with sodium borohydride at room temperature in ether and then converted to the corresponding
 20 chloroformate with triphosgene as described above.

Preferred compounds of formula (IV) where Q is -O-(N-succinimide) are the novel compounds of formula (XVI):



- wherein R_5 represents C_{1-6} alkyl, preferably ethyl or t-butyl. Therefore, according to a further aspect of the present invention
 25 there is provided compounds of formula (XVI). These compounds may be prepared according to the method disclosed in Example 2 or analogous methods.

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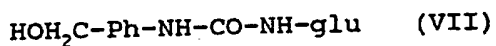
SCHEME 1

SUBSTITUTE SHEET (RULE 26)

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Compounds of the formula (V) and (VI) may be made from ras inhibitors which contain an amino or hydroxy group by analogous procedures to the methods described above for the production of compounds of formulae (II) and (III).

- 5 Ras inhibitor prodrugs of the formulae (V) or (VI) will be activated by carboxypeptidases such as CPG2 by the action of the enzyme to remove the glutamic acid residue followed by "self immolation" of the remaining prodrug in a manner analogous to that described above in relation to the nitroreductases.
- 10 Prodrugs of the formula (V) where Z is -NH.CO- may also be made using linkers of the formula (VII):



- where Ph and glu are as defined above. The optionally substituted phenylene group is substituted at the 4-position by
- 15 the glu-containing moiety relative to the hydroxymethyl group.

The linkers of formula (VII) may be made from optionally substituted 4-nitrobenzyl alcohol, where the optional substituents are as defined for the group -Ph- above. The hydroxyl group of the 4-nitrobenzyl alcohol is protected, for

20 example by reaction with tert-butyl-diphenyl-chloro-silane at room temperature in an organic solvent, to provide an optionally substituted (4-nitro-benzyl) tert-butyl-di-phenyl-silyl ether. The 4-nitro group is then reduced to an amine group by catalytic hydrogenation or catalytic hydrogen transfer, for example with

25 ammonium formate in the presence of a catalyst such as Pd/C in a protic solvent such as an alcohol, e.g. methanol or ethanol.

The amine group may then be converted into an isocyanate group for example by reaction with phosgene, diphosgene or triphosgene in the presence of a tertiary organic amine such as triethylamine

30 and an aprotic organic solvent with a boiling point higher than 50°C such as toluene. The isocyanate compound is then reacted with di-C₁₋₆alkyl-glutamic acid or derivative thereof, eg di-C₁₋₆alkyl-glutamate hydrochloride. This may be done at room

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temperature in the presence of triethylamine in an aprotic organic solvent such as toluene, THF or dichloromethane.

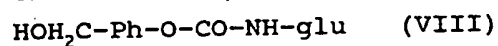
Alternatively, the amine compound may be reacted directly in a one-pot synthesis with the di-C₁₋₆alkyl-glutamic acid or derivative thereof in the presence of triphosgene and triethylamine in an aprotic solvent such as THF or dichloromethane.

In either case, the resulting compound is treated to remove the hydroxy-protecting group, for example by the use of tetra-butyl- ammonium fluoride in THF at room temperature.

The resulting compound of formula (VII) where glu is in the form of a di-C₁₋₆alkyl ester may be deprotected to remove the ester groups for example by the use of an acid such as formic or trifluoro acetic acid. Compounds of formula (VII) or (XVI) may be linked to a FTLi containing a group -OH, -NH₂ or -SH by reaction with the FTLi or activated derivative thereof in aprotic solvents such as dichloromethane and/or THF in the presence of a tertiary organic base such as triethylamine at room temperature, to provide a compound of the formula (V). The di-C₁₋₆alkyl ester groups of the compound, if present, may be removed as described above.

In order to link a FTLi with a group -XH to the linker of formula (VII) the group -XH may be converted to a reactive chloroformyl, chlorothioformyl or isocyanate derivative by the use of phosgene, diphosgene or triphosgene in the presence of a phase transfer catalyst such as tetra-butyl ammonium hydrogen sulphate. The reaction may be carried out in the presence of a base such as NaOH in an organic solvent such as toluene, THF or dichloromethane.

Prodrugs of the formula (V) in which Z is -O.CO- may be made using linkers of the formula (VIII):



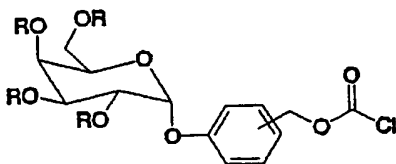
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where Ph and glu are as defined above. The optionally substituted phenylene group is substituted at the 4-position by the glu-containing moiety relative to the hydroxymethyl group.

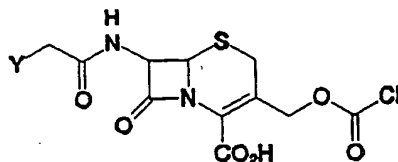
To produce a compound of formula (VIII), optionally substituted 4-hydroxybenzaldehyde is protected as a 1,3-dithiane or dithiolanein in an aprotic solvent such as CH_2Cl_2 in the presence of $\text{BF}_3 \cdot \text{Et}_2\text{O}$, at room temperature by reaction with 1,3-propanedithiol or 1,2-ethanedithiol, to give the corresponding 4(1',3'-dithianyl) phenol or 4(1',3'-dithidanyl) phenol. This compound is coupled with di- C_{1-6} alkyl-glutamyl isocyanate, in an aprotic solvent such as toluene in the presence of a tertiary organic amine such as triethylamine, to the corresponding O[4(1',3'-dithianyl)-phenyl]N(di- C_{1-6} alkyl-glutamyl)carbamate. The deprotection of the carbamate to the corresponding aldehyde, may be carried out with $\text{Hg}(\text{ClO}_4)_2$ or $\text{Tl}(\text{NO}_3)_3$ in THF or dichloromethane at room temperature. The reduction of the aldehyde yields the desired O(4-benzyl-oxy)N(di- C_{1-6} alkyl-glutamyl) carbamate. This may be deprotected by treatment with an acid such as trifluoroacetic or formic acid to remove the alkyl ester protecting groups to provide a prodrug of formula (V).

The linkers of formula (VIII) may be attached to FTLi compounds containing a free hydroxy, amino or mercapto group in the same way as described above for the linkers of formula (VII) or (XVI).

Other suitable FTLi prodrugs include those which are derivatized with a sugar or a β -lactam derivative. For example, suitable linkers which may be attached to FTL inhibitors of the type FTL- NH_2 or FTL-OH or FTL-SH described above are:



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where R is hydrogen or acetyl and Y is aryl such as phenyl, benzyl or tolulyl, and these may be made in an analogous manner to the other prodrugs described above.

Any hydroxy, amino or mercapto group of a FTLi may be linked in the manner described above to provide a prodrug of the present invention. If desired, more than one such group may be derivatized to make a prodrug. If however only a single hydroxy, mercapto or amino group is to be reacted to form a prodrug, any remaining groups of the FTL may be protected with for example t-butyl or adamantyl groups (in the case of hydroxyl) or butyloxycarbonyl groups in the case of amino. Such protecting groups may be attached using chemical processes known in the art. The groups of the FTLi to be reacted with the linker may be derivatized to the corresponding haloformate or isocyanate and then coupled with the linkers such as those of formulae (VII) or (VIII). After the FTLi prodrug has been made, the protecting groups may be removed by conventional means, eg by treatment with trifluoroacetic acid.

Physiologically acceptable derivatives of said prodrug include salts, amides, esters and salts of esters. Esters include carboxylic acid esters in which the non-carbonyl moiety of the ester grouping is selected from straight or branched chain C_{1-6} alkyl, (methyl, n-propyl, n-butyl or t-butyl); or C_{3-6} cyclic alkyl (e.g. cyclohexyl). Salts include physiologically acceptable base salts, eg derived from an appropriate base, such as alkali metal (e.g. sodium), alkaline earth metal (e.g. magnesium) salts, ammonium and NR_4 (wherein R is C_{1-4} alkyl)

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salts. Other salts include acid addition salts, including the hydrochloride and acetate salts. Amides include non-substituted and mono- and di-substituted derivatives.

The invention further provides pharmaceutical formulations. Such
5 formulations comprise a compound of the invention together with one or more pharmaceutically acceptable carriers or diluents.

Pharmaceutically acceptable carriers or diluents include those used in formulations suitable for oral or parenteral (e.g. intramuscular or intravenous) administration. The formulations
10 may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. Such methods include the step of bringing into association the active ingredient with the carrier which constitutes one or more accessory ingredients. In general the formulations are prepared
15 by uniformly and intimately bringing into association the active ingredient with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

For example, formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which
20 may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents, and liposomes or other microparticulate systems which are
25 designed to target the polypeptide to blood components or one or more organs.

Suitable liposomes include, for example, those comprising the positively charged lipid (N[1-(2,3-dioleoyloxy)propyl]-N,N,N-triethylammonium (DOTMA), those comprising dioleoylphosphatidyl-
30 ethanolamine (DOPE), and those comprising 3β [N-(n',N'-dimethyl-aminoethane)-carbamoyl]cholesterol (DC-Chol).

The ras inhibitor prodrugs of the present invention and the

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antibody/enzyme conjugate for ADEPT can be administered simultaneously but it is often found preferable, in clinical practice, to administer the enzyme/agent conjugate before the prodrug, e.g. up to 72 hours or even 1 week before, in order to
5 give the enzyme/agent conjugate an opportunity to localise in the region of the tumour target. By operating in this way, when the prodrug is administered, conversion of the prodrug to the cytotoxic agent tends to be confined to the regions where the enzyme/agent conjugate is localised, i.e. the region of the
10 target tumour and the premature release of the ras inhibitor agent is minimised.

In GDEPT the prodrug will usually be administered following administration of the modified virus encoding an enzyme. Typically, the virus will be administered to the patient and then
15 the uptake of the virus by infected cells monitored, for example by recovery and analysis of a biopsy sample of targeted tissue. Alternatively, the prodrug may be administered following the administration of a delivery system containing the gene encoding the enzyme.

20 In ADEPT the degree of localisation of the enzyme/agent conjugate (in terms of the ratio of localized to freely circulating active conjugate) can be further enhanced using the clearance and/or inactivation systems described in WO89/10140. This involves, usually following administration of the conjugate and before
25 administration of the prodrug, the administration of a component (a "second component") which is able to bind to the such part of the conjugate so as to inactivate the enzyme and/or accelerate the clearance of the conjugate from the blood. Such a component may include an antibody to the enzyme component of the system
30 which is capable of inactivating the enzyme.

The second component may be linked to a macromolecule such as dextran, a liposome, albumin, macroglobulin or a blood group O erythrocyte so that the second component is restrained from leaving the vascular compartment. In addition or as an

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alternative, the second component may include a sufficient number of covalently bound galactose residues, or residues of other sugars such as lactose or mannose, so that it can bind the conjugate in plasma but be removed together with the conjugate from plasma by receptors for galactose or other sugars in the liver. The second component should be administered and designed for use such that it will not, to any appreciable extent, enter the extravascular space of the tumour where it could inactivate localised conjugate prior to and during administration of the prodrug.

The exact dosage regime for both GDEPT and ADEPT will, of course, need to be determined by individual clinicians for individual patients and this, in turn, will be controlled by the exact nature of the prodrug and the cytotoxic agent to be released from the prodrug but some general guidance can be given. Chemotherapy of this type will normally involve parenteral administration of both the prodrug and either the enzyme/agent conjugate or modified virus and administration by the intravenous route is frequently found to be the most practical. In ADEPT systems, the dose of the prodrug and conjugate will ultimately be at the discretion of the physician, who will take into account such factors as the age, weight and condition of the patient. Suitable doses of prodrug and conjugate are given in Bagshawe et al. Antibody, Immunoconjugates, and Radiopharmaceuticals (1991), 4, 915-922. A suitable dose of conjugate may be from 500 to 200,000 enzyme units/m² (e.g. 20,000 enzyme units/m²) and a suitable dose of prodrug may be from 5 to 2000 mg/m² (e.g. 200 mg/m²).

In order to secure maximum concentration of the conjugate at the site of desired treatment, it is normally desirable to space apart administration of the two components by at least 4 hours. The exact regime will be influenced by various factors including the nature of the tumour to be targeted and the nature of the prodrug, but usually there will be an adequate concentration of the conjugate at the site of desired treatment within 48 hours.

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In GDEPT systems, the amount of virus or other vector delivered will be such as to provide a similar cellular concentration of enzyme as in the ADEPT system mentioned above. This may be determined by clinical trials which involve administering a range of trial doses to a patient and measuring the degree of infection or transfection of a target cell or tumour. The amount of prodrug required will be similar to or greater than that for ADEPT systems.

The present invention also provides a system for use in the control of neoplasia in a human or animal subject comprising an enzyme capable of converting a ras inhibitor prodrug to an active ras inhibitor, preferably conjugated with a targeting agent such as monoclonal antibody that will bind to a tumour-associated antigen, in association with a ras inhibitor prodrug as defined above. When the enzyme is a nitroreductase, the system also preferably comprises a suitable cofactor for the enzyme. Suitable cofactors include a riboside or ribotide of nicotinic acid or nicotinamide.

The present invention extends to a method of treating neoplasia in a human or animal subject requiring such treatment which comprises administering to the host an effective amount of a ras inhibitor prodrug of the invention and an enzyme, preferably conjugated with a targeting agent such as a monoclonal antibody that will bind to a tumour-associated antigen. Tumour-associated antigens include tumour-associated receptors present on the cell surface.

The present invention also provides a system for use in the control of neoplasia in a human or animal subject comprising a modified virus capable of selectively infecting tumour cells in said subject, said virus carrying a DNA or RNA sequence encoding an enzyme, in association with a ras inhibitor prodrug capable of being converted to a ras inhibitor by the action of said enzyme.

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The present invention extends to a method of treating neoplasia in a human or animal subject requiring such treatment which comprises administering to the host an effective amount of a ras inhibitor prodrug of the invention and a modified virus, said
5 modified virus capable of selectively infecting tumour cells in said subject, said virus carrying a DNA or RNA sequence encoding an enzyme capable of converting said ras inhibitor prodrug to a ras inhibitor.

The present invention also extends to a method of treating
10 neoplasia in a human or animal subject requiring such treatment which comprises administering to the host an effective amount of a ras inhibitor prodrug of the invention and a non viral vector system, said non-viral vector system capable of being selectively introduced into tumour cells in said subject, said vector system
15 carrying a DNA or RNA sequence encoding an enzyme capable of converting said ras inhibitor prodrug to an active ras inhibitor operably linked to a promoter effective in expressing said enzyme in said cells.

The various systems for use in the treatment of neoplasia by
20 ADEPT described above optionally include the "second component" for accelerated clearance described above. Likewise, the methods of treatment of neoplasia described above optionally include as part of that method the use of the second component, an effective amount of which is administered after administration of the
25 enzyme, in order to increase the ratio of localised to freely circulating enzyme. Reference may be made to WO89/10140 for further particular details of the second component, and such details can be incorporated for use in the present invention.

Modified viruses capable of selectively infecting tumour cells
30 are known in the art. By "selectively infecting" it is meant that the virus will primarily infect tumour cells and that the proportion of non-tumour cells infected is such that the damage to non-tumour cells by administration of the ras inhibitor prodrug will be acceptably low, given the nature of the disease

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being treated. Ultimately, this will be determined by the physician.

It will also be understood that the DNA or RNA sequence encoding an enzyme carried by the virus will be linked to suitable expression control signals such that expression of the enzyme will occur in the targeted tumour cells.

The non-viral vector system will be capable of being selectively introduced into tumour cells utilizing methods such as those mentioned above, e.g. calcium phosphate co-precipitation, microinjection, liposomes, direct DNA uptake, and receptor-mediated DNA transfer (Morgan & French Anderson, Annu. Rev. Biochem., 1993, 62;191).

Suitable monoclonal antibodies for use in the present invention include antibodies to cerbB2, such as ICR12 (Bakir, M A et al, J. Nucl. Med (1992) 33;2154-2160), and antibodies to epidermal growth factor receptor, such as ICR16 (Dean, CJ et al, Int. J. Cancer Suppl. 8, (1994), 103). Evidence that cerbB2 requires ras for transformation is given in Ben-Levy et al, Embo J. 13,3302,1994.

As used herein, the term "monoclonal antibody" will be understood by those of skill in the art not simply to refer to antibodies produced by traditional hybridoma techniques, but also to cover antibodies and variants thereof produced by recombinant means. These include, for example, humanised antibodies such as those with a constant region from a human antibody grafted onto a non-human antibody variable region (see for example EP-A-O 120 694), chimeric antibodies such as those with non-human complementarity determining regions (CDRs) grafted into a human variable region framework (see for example EP-A-O 239 400) and single chain antibodies. Fragments of such monoclonal antibodies which retain their target binding activity are also included by the general term "monoclonal antibody". This includes Fv, Fab' and F(ab')₂ fragments. It also includes recombinant or synthetic proteins

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based upon the CDRs of such antibodies, e.g. abzymes (a polypeptide with both antibody-like binding activity and enzyme activity) and diabodies.

Prodrugs of the present invention may also be used as reagents
5 in in vitro systems to test the activity of candidate enzymes or antibodies which may be incorporated into ADEPT or GDEPT systems.

For example, a tumour cell line carrying a marker to which an antibody is directed may be grown in vitro, and then an antibody-enzyme conjugate added to the culture. The enzyme will be one
10 which is, or suspected to be, capable of converting a prodrug of the invention into an active drug. The prodrug is then added to the culture and the amount of cell killing or inhibition of cell growth is measured (for example by using a vital stain to record the number of viable cells or by replating a sample of the
15 culture to count the number of viable cells).

EXAMPLES

The following Examples 1 and 2 further illustrate the invention. Preparative example 1 is an illustration of the preparation of a compound of formula (Ib). The reaction schemes which follow
20 further illustrate the Examples.

All starting materials, reagents and anhydrous solvents (THF under N₂) were purchased from Aldrich, unless otherwise stated. TLC was performed on precoated sheets of Kieselgel 60 F₂₅₄ (Art 5735, Merck). Preparative TLC was carried out on Silica Gel
25 plates (20 x 20 cm) from Analtech. NMR spectra were determined in Me₂SO-d₆ on a Bruker AC250 spectrometer (250 MHz) at 30°C (303K) unless otherwise stated. Coupling constants (J) were expressed in Hz.

PREPARATIVE EXAMPLE 1

30 N-methoxy-N-methyl Amide of N-t-butoxycarbonyl Isoleucine (I)

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O,N,-Dimethylhydroxylamine hydrochloride (976 mg, 10 mmol) was suspended in dichloromethane (10ml) and cooled to -10°C. 1-methylpiperidine (992 mg, 1.22 ml, 10 mmol) was then added slowly, keeping the temperature below -2°C. This solution was
5 stored at -10°C. In a separate flask N-boc isoleucine (2.290g, 9.9 mmol) was dissolved in dichloromethane (40ml) and cooled to -20°C. 1-methylpiperidine (992 mg, 1.22 ml, 10 mmol) and isobutylchloroformate (1.336 g, 1.30 ml, 10 mmol) were then added keeping the temperature below -20°C. After 15 min the
10 hydroxylamine solution prepared above was added in a single portion and the solution stirred at ambient temperature overnight. The solution was then washed with saturated ammonium chloride solution (2 x 30 ml), dried over magnesium sulfate, filtered and the solvent removed under reduced pressure to yield
15 a yellow oil which was purified by column chromatography (silica - 10-30% ethylacetate in hexane) to yield the N-methoxy-N-methyl amide (2.28 g, 84%) as a colourless oil.

The yields for the subsequent preparations of this compound varied between 60 and 84%.

20 N-t-Butoxycarbonyl isoleucinal(2)

The Weinreb amide of N-t-butoxycarbonylisoleucine, prepared above, (1.98 g, 7.2 mmol) was dissolved in anhydrous ether (10 ml) and cooled to -45°C. Lithium aluminium hydride (10 ml of a 1N solution in ether, 10 mmol) was then added keeping the
25 temperature below -35°C, after addition the solution was then allowed to warm to 5°C. The solution was then cooled to -45°C and sodium sulfate decahydrate (5.00 g) added and the cooling bath removed. After 1 hour the solution was filtered through celite and the solvent removed under reduced pressure to yield
30 the aldehyde (1.47 g, 95%) as a colourless oil, which was immediately used in the next reaction as it racemises at room temperature.

The yields for the subsequent preparations of this compound varied between 89 and 97%.

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N-[2(S)-[t-butoxycarbonylamino]-3-methylpentyl]-isoleucine (3)

Isoleucine (1.31 g, 9.95 mmol) was suspended in anhydrous methanol (10 ml) and trifluoroacetic acid (1.133 g, 0.77 ml, 9.95 mmol) was added. The mixture was stirred until all the
5 isoleucine had dissolved and the solvent was then removed under reduced pressure. Anhydrous DMF (10ml) was added to the resulting isoleucine trifluoroacetate salt followed by 3Å molecular sieves and a solution of 2 (1.47 g, 4.9 mmol) in anhydrous DMF (5 ml). After 15 minutes the solution was treated
10 with sodium triacetoxy borohydride (1.58 g, 7.45 mmol) and stirred overnight. The resulting suspension was filtered through celite, diluted with ethyl acetate (50 ml) and washed with saturated sodium chloride solution (2 x 35 ml). The organic
15 extracts were dried over magnesium sulfate, filtered, and the solvent removed under reduced pressure to yield the acid (1.36 g, 84%) as a yellow oil.

The yields for the subsequent preparations of this compound varied between 67 and 88%.

N-2(S)-[t-butoxycarbonylamino-3-methylpentyl]-isoleucylhomoserine20 lactone (4)

Compound 3 (331 mg, 1 mmol), dissolved in anhydrous DMF (2ml) was treated with hydroxybenzotriazole hydrate (135 mg, 1 mmol), 1-(3-dimethylaminopropyl)-3-ethyl carbodimide hydrochloride (260 mg, 1.36 mmol) and homoserine lactone hydrochloride (125 mg, 1 mmol).
25 Triethylamine (174 mg, 0.24 ml, 1.72 mmol) was added and the mixture stirred overnight. The solution was diluted with ethyl acetate (25 ml) and washed with saturated sodium chloride solution (2 x 10ml), dried over magnesium sulfate, filtered and the solvent removed under reduced pressure to yield a yellow oil.
30 This was then purified by column chromatography (5% methanol in dichloromethane) and by precipitation from ether by the dropwise addition of hexane, to give the product as a white powder (147 mg, 47%).

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The yields for the subsequent preparations of this compound varied between 41 and 54%; m/z: [M+H]⁺=414; microanalysis data:

	Expected %	Found %
C	60.99	60.7
H	9.51	9.8
N	10.16	9.9

N-[2(S)-Amino-3-methylpentyl]-isoleucylhomoserine lactone hydrochloride (5)

Compound 4 (147 mg, 0.35 mmol) was dissolved in ethyl acetate (10ml) and cooled to -25°C. Anhydrous hydrogen chloride gas was then passed through the solution until tlc (5% methanol in dichloromethane) showed complete reaction. Nitrogen was then passed through the solution to remove excess hydrogen chloride. The solvent was then removed under reduced pressure to yield the product as a pale yellow solid (125 mg, 91%).

The yields for the subsequent preparations of this compound varied between 62 and 92%.

N-methoxy-N-methyl amide Of N-t-Butoxycarbonyl-S-Trityl-cysteine (6)

O,N-dimethylhydroxylamine hydrochloride (108 mg, 1.1 mmol) was suspended in dichloromethane (10ml) and cooled to -10°C. 1-methylpiperidine (110 mg, 0.13 ml, 1.1 mmol) was then added slowly, keeping the temperature below -2°C. This solution was stored at -10°C. In a separate flask N-boc-S-trityl cysteine (464 mg, 1 mmol) was dissolved in dichloromethane (20 ml) and cooled to -20°C. 1-methylpiperidine (110 mg, 0.13 ml, 1.1 mmol) and isobutylchloroformate (144 mg, 0.14 ml, 1.1 mmol) were then added keeping the temperature below -20°C. After 15 min the hydroxylamine solution prepared above was added in a single portion and the solution stirred at ambient temperature overnight. The solution was then washed with saturated ammonium

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chloride solution (2 x 15 cm³), dried over magnesium sulfate, filtered and the solvent removed under reduced pressure to yield a white foam which was purified by column chromatography (silica - 10-30% ethyl acetate in hexane) to yield the N-methoxy-N-methyl amide (406 mg, 81%) as a white foam.

The yields for the subsequent preparations of this compound varied between 81 and 92%.

N-t-butoxycarbonyl-S-trityl-Cysteinal (7)

The Weinreb amide of N-t-butoxycarbonyl-S-tritylcysteine, prepared above, (406 mg, 0.8 mmol) was dissolved in anhydrous ether (5ml) and cooled to -45°C. Lithium aluminium hydride (1ml of a 1 N solution in ether, 1 mmol) was then added keeping the temperature below -35°C, after addition the solution was then allowed to warm to 5°C. The solution was then cooled to -45°C and sodium sulfate decahydrate (1.25g) added and the cooling bath removed. After 1 hour the solution was filtered through celite and the solvent removed under reduced pressure to yield the aldehyde (332 mg, 93%) as a white foam.

The yields for the subsequent preparations of this compound varied between 84 and 93%.

N-[2(S)-2-((t-butoxycarbonyl)amino)-3-[(triphenylmethyl)thio]propyl]amino[3-methylpentyl]isoleucylhomoserine lactone (8).

Compound 5 (125 mg, 0.32 mmol) was dissolved in anhydrous methanol (10ml) and treated with 3A molecular sieves, potassium acetate (64 mg, 0.64 mmol), N-t-butoxycarbonyl-S-trityl cysteinal (289 mg, 0.64 mmol) and sodium cyanoborohydride (31 mg, 0.48 mmol). The mixture was stirred at ambient temperature for 24 hours, filtered through celite and the methanol removed under reduced pressure. The residue was dissolved in ethyl acetate (25ml), washed with saturated ammonium chloride solution (15ml) and

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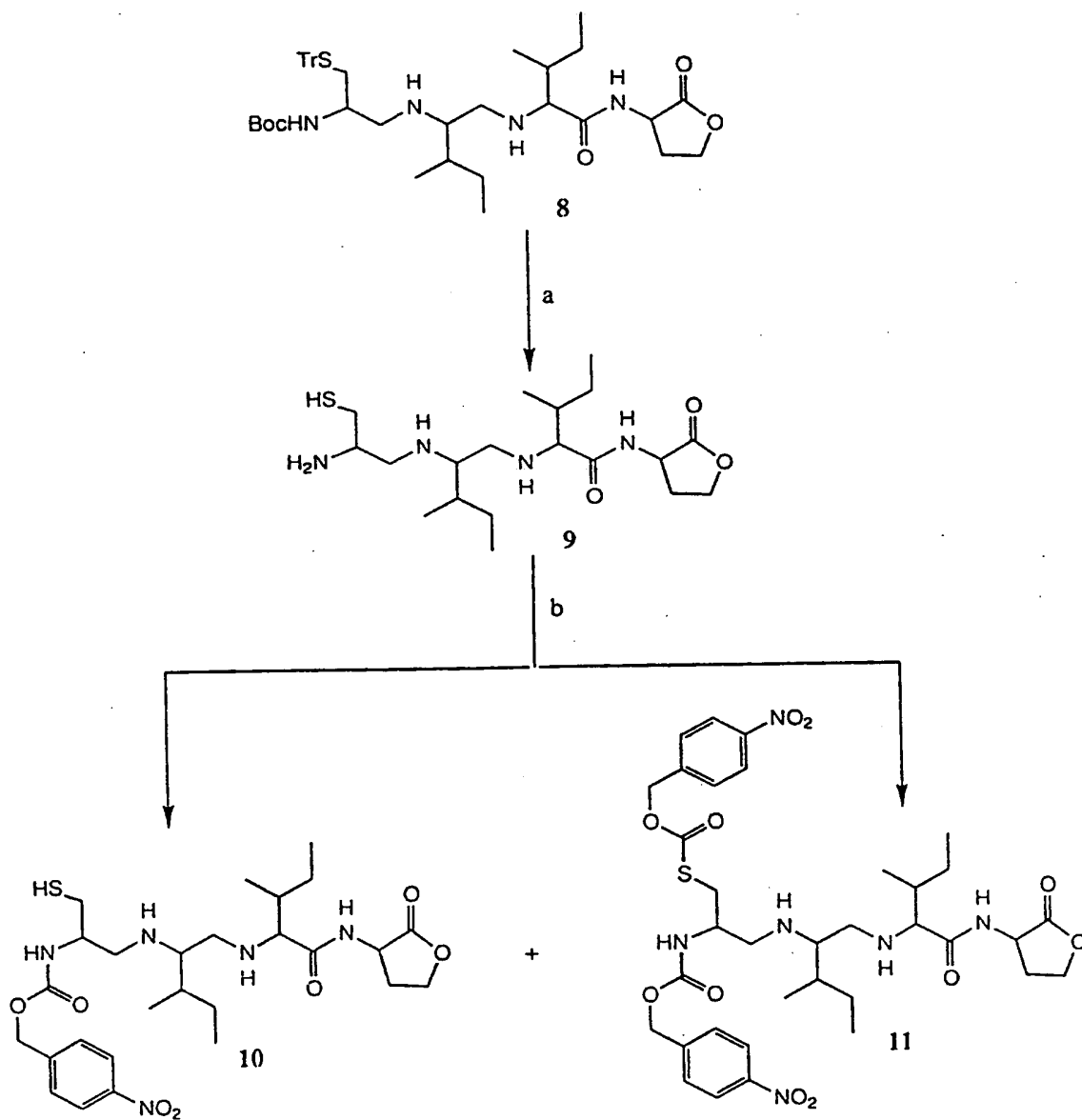
saturated sodium chloride solution (15ml), dried over magnesium sulfate, filtered and the solvent removed under reduced pressure to yield a white foam. This was then purified by column chromatography (silica, 1-3% methanol in dichloromethane) to
5 yield the desired compound (107 mg, 45%) as a white foam.

The yields for the subsequent preparations of this compound were 32 and 43%; m/z : $[M+H]^+ = 745$.

EXAMPLE 1

SUMMARY

10 Two ras inhibitor prodrugs which may be activated by nitroreductase were made. These are N-(2(R)-[[2(4'-nitrobenzyloxy-carbonyl)-amino-3-mercaptopropyl]amino]-3-methyl-pentyl]isoleucylhomoserine lactone, 10 and N(2(R)-[[2(4'-nitrobenzyloxy-carbonyl)-amino]-3[(4'-nitrobenzyloxy-carbonyl)-
15 thio]propyl]amino]-3-methyl-pentyl]isoleucylhomoserine lactone, 11 (see scheme 2). The starting material was N-(2(S)-2-[(tert-butoxy-carbonyl)-amino-3[(triphenylmethyl)thio]propyl]amino)-3-methyl-pentyl]isoleucylhomoserine lactone, 8, which was the product of Preparative Example 1. It was deprotected by using
20 triethylsilane and TFA in CH_2Cl_2 (according to Graham *et al.* (J. Med. Chem., 1994, 37(6), 725-732) procedure with the sole difference that the reaction was carried out at 4°C). After work up (but no final purification by HPLC), the compound 9, thus obtained was reacted with 4-nitro-benzyl chloroformate to give
25 a mixture of compounds 10 and 11 which were separated and purified by preparative TLC. The disappearance from the 1H -NMR spectrum of compound 11, of the proton belonging to the mercapto group (range 1.0-2.0 ppm) supported the location of the second nitro-benzylic moiety.



SCHEME 2

- 30 -

N-(2(R)-[2(-amino-3-mercaptopropyl)amino]-3-methyl-pentyl]isoleucylhomoserine lactone 9

N-(2(S)-2-[tert-butoxycarbonyl]amino-3[(triphenylmethyl)-thio]propyl]amino]-3-methyl-pentyl]isoleucylhomoserine lactone, 8, (0.200 g, 0.148 mmol) was dissolved in CH_2Cl_2 (2.0 ml) with TFA (1.0 ml) and triethylsilane (0.18 ml, 0.13 g, 1.1 mmol) at 4°C. The mixture was stirred at this temperature for 2 h, then allowed to reach the ambient temperature and concentrated to dryness. The residue was partitioned between hexane fraction and aqueous TFA 0.1% (6.0 ml). The aqueous layer was washed with hexane fraction (2x2.5 ml), filtered and concentrated to dryness at 20-25°C. An oily residue (0.257 g) was obtained which was used without further purification.

N-(2(R)-[[2(4'-nitro-benzyloxy-carbonyl)-amino-3-mercaptopropyl]amino]-3-methyl-pentyl]isoleucylhomoserine lactone, (10) and N-(2(R)-[[2(4'-nitro-benzyloxy-carbonyl)-amino]-3[(4'-nitro-benzyloxy-carbonyl)-thio]propyl]amino]-3-methyl-pentyl]isoleucylhomoserine lactone 11

To a stirred solution of the previous lactone product, 9, (0.128g) in THF (dry) (2 ml) and triethylamine (0.12 ml, 0.081 g, 0.8 mmol), was added 4-nitro-benzyl chloroformate (0.047 g, 0.22 mmol). After 5 h, the reaction mixture was evaporated to an oil (0.175 g). The oil was partitioned between EtOAc (3 ml) and H_2O (2 ml). The organic layer was washed with H_2O (2x2 ml), dried (MgSO_4) and evaporated under vacuum. An oil (0.070 g) resulted containing a mixture of 10 and 11. The compounds were purified by preparative TLC. Eluent: EtOAc: cyclohexane 3:1.

10 eluted first: 0.005 g yield = 33%. $^1\text{H-NMR}$, d_H : 0.73-0.90 (12H, m), 1.00-1.22 (3H, m, $2\text{H}+\text{H}_S$), 1.25 (2H, d, $J = 7.44$), 1.40-1.52 (3H, m), 2.10-2.18 (1H, m), 2.30-2.82 (5H, m), 2.90-3.10 (1H, m), 4.00-4.10 (1H, m), 4.15-4.27 (1H, m), 4.30-4.42 (1H, m), 4.50-4.65 (1H, m), 5.40 (2H, s, CH_2 -benzyl), 7.63 (2H, d, $J = 8.70$, $\text{H}_{\text{arom}2+6}$), 8.24 (2H, d, $\text{H}_{\text{arom}3+5}$); $\text{C}_{27}\text{H}_{43}\text{N}_5\text{O}_7\text{S}$.

11 eluted later: 0.003 g yield = 15%. $^1\text{H-NMR}$, d_H : 0.70-0.92 (12H, m), 1.03-1.20 (2H, m), 1.25 (2H, d, $J = 6.12$), 1.40-1.60 (3H, m),

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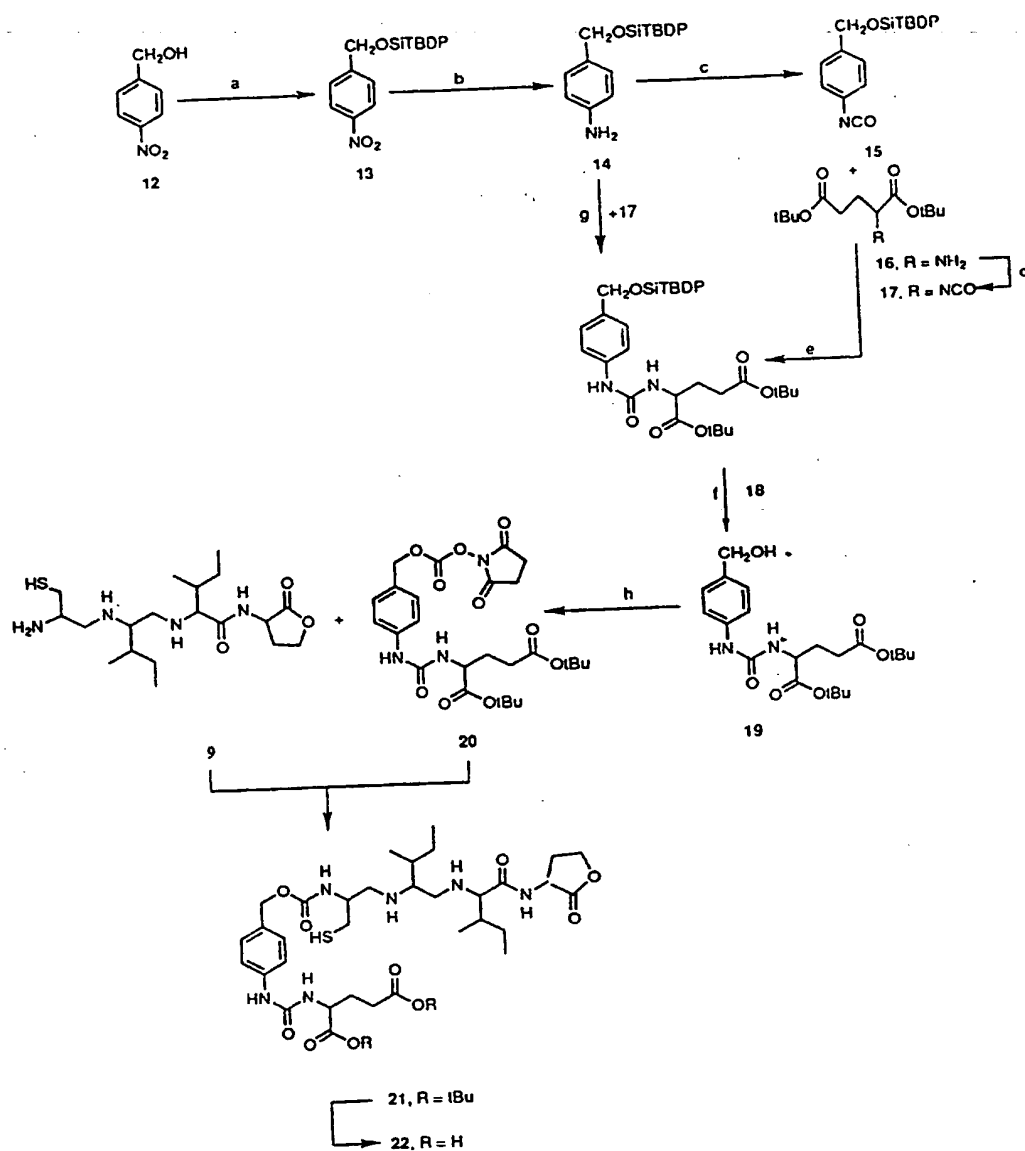
2.16-2.24 (1H, m), 2.25-2.82 (5H, m), 2.82-2.92 (1H, m), 3.64-3.80 (1H, m), 4.12-4.26 (1H, m), 4.27-4.43 (1H, m), 4.50-4.65 (1H, m), 5.16 (2H, s, CH₂-S-benzyl), 5.39 (2H, s, CH₂-N-benzyl), 7.59 (2H, d, J = 8.30, H_{arom2+6}, S-PhNO₂), 7.61 (2H, d, J = 8.46, H_{arom2+6}, N-PhNO₂), 8.20 (2H, d, H_{arom3+5}, S-PhNO₂), 8.21 (2H, d, H_{arom3+5}, N-PhNO₂); C₃₅H₄₈N₆O₁₁S.

EXAMPLE 2

SUMMARY

For the synthesis of a novel ras prodrug cleavable by the enzyme CPG2, an ester of a self-immolative linker was designed to be coupled to mercapto or amino functional groups. This is: N¹(4'-succinimidyl-oxycarbonyl-oxybenzyl)N³(di-tert-butyl-glutamyl)urea, 20 (see scheme 3). Once coupled to the ras inhibitor, the ester protecting groups are removed. The route to the ras prodrug is exemplified.

The intermediate N¹(4-hydroxybenzyl)N³(di-tert-butyl-glutamyl)urea 19, was synthesised for coupling to ras inhibitor drugs according to the Scheme 2. The starting material, 4-nitrobenzyl alcohol, 12, was protected as tert-butyl-di-phenyl-silyl ether, 13, by reacting with tert-butyl-diphenyl-chlorosilane and imidazole in DMF (or THF) at room temperature. The protected nitro derivative, 13, was reduced by hydrogen transfer with ammonium formate (Pd/C 10% in EtOH). The amine, 14, thus formed was reacted with triphosgene in toluene at 70 °C, to form the corresponding isocyanate 15. The protected linker, 18, was obtained by coupling the isocyanate 15 with di-tert-butyl-glutamate in THF in the presence of NEt₃ at room temperature. An alternative route to 18 was by the direct coupling of amine 14 with the di-tert-butyl-glutamyl isocyanate 17, under the same conditions as described above, where the di-tert-butyl-glutamyl isocyanate 17 was obtained from the di-tert-butyl-glutamate by treatment with triphosgene and NEt₃ in toluene at -78 °C. Using this route, the compound 18 was obtained in good yield from the amine 14 and di-tert-butyl-glutamate in a one-pot synthesis.

Ras linker for CPG-2)

SCHEME 3

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The compound 18 was deprotected by Bu_4NF in THF at room temperature and the di-tert-butyl ester of the linker, 19, was purified by column chromatography. The compound 19 was reacted with the disuccinimidyl carbonate in acetonitrile and triethylamine at room temperature leading to the desired succinimidyl linker, 20. This is then coupled with the ras inhibitor, 9 (prepared according to Preparative Example 1 and Example 1), giving the compound, 21, which is then deprotected in formic acid at 4°C to give the final ras prodrug, 22.

10 EXPERIMENTAL

(4-nitro-benzyl) tert-butyl-di-phenyl-silyl ether (13).

To a stirred solution of 4-nitrobenzyl alcohol, 12, (1.00 g, 6.50 mmol), and imidazole (0.97 g, 14.1 mmol) in DMF (10.0 ml), was added tert-butyl-diphenyl-chlorosilane (1.98 g, 7.20 mmol) over 10 min under N_2 at room temperature. The reaction mixture was stirred for an additional 5 h, diluted with Et_2O (75 ml), washed with H_2O (5 x 15 ml), dried (MgSO_4) and evaporated to dryness under vacuum. An oil was obtained which crystallised on standing and was recrystallised to a solid from EtOH (70%); yield: 2.36 g (93%). $\nu_{\text{max}}/\text{cm}^{-1}$ (film): 2931, 2857 (CH_2 , asym., sym.), 1521, 1345 (NO_2); $^1\text{H-NMR}$, d_H : 1.06 (9H, s, t-Bu), 4.92 (2H, s, CH_2), 7.42-7.46 (5H, m, Ph), 7.63-7.65 (7H, m, $\text{Ph} + \text{H}_{\text{arom2+6}}$), 8.23 (2H, d, $J = 8.23$, $\text{H}_{\text{arom3+5}}$); MS, (EI), (391.54); m/z : 334 ($\text{M} - \text{t-Bu}$, 100), 288 ($\text{M} - \text{t-Bu} - \text{NO}_2$, 10), 256 ($\text{M} - \text{t-Bu} - \text{Ph}$, 20), 199 (Ph_2SiOH^+ , 100); $\text{C}_{23}\text{H}_{25}\text{NO}_3\text{Si}$.

(4-amino-benzyl) tert-butyl-di-phenyl-silyl ether (14)

To a stirred solution of 13 (5.00 g, 12.77 mmol) in ethanol (100 ml) was added Pd/C (10%, 1.50 g) and ammonium formate (4.60 g) at room temperature. After 1.5 h the catalyst was removed by filtration, the filtrate concentrated to dryness under vacuum and the residue partitioned between $\text{EtOAc}:\text{H}_2\text{O}$. The organic layer was dried (MgSO_4) and concentrated under vacuum to give 14 as an oil;

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yield: 4.24 g (92 %); $\nu_{\max}/\text{cm}^{-1}$ (film): 3433, 3378 (NH_2), 2931, 2857 (CH_2 , asym., sym.); $^1\text{H-NMR}$, d_H : 1.00 (9H, s, $t\text{-Bu}$), 4.57 (2H, s, CH_2), 4.98 (2H, s broad, NH_2), 6.52 (2H, d, $J = 8.25$, $\text{H}_{\text{arom}3+5}$), 6.96 (2H, d, $\text{H}_{\text{arom}2+6}$), 7.42-7.46 (5H, m, Ph), 7.62-7.65 (5H, m, 5 Ph); MS, (EI), (361.56); m/z : 361 (M^+ , 8), 304 ($\text{M} - t\text{-Bu}$, 100), 199 (Ph_2SiOH^+ , 100); $\text{C}_{23}\text{H}_{27}\text{NOSi}$.

(4-isocyanato-benzyl)tert-butyl-di-phenyl-silyl ether (15)

To a stirred solution of 14 (0.63 g, 1.70 mmol) and triethylamine (0.16 g, 0.60 mmol) in toluene (10 ml) at 70 $^{\circ}\text{C}$, was added
 10 triphosgene (0.18 g, 1.7 mmol). After 5 h the reaction mixture was filtered and the filtrate evaporated to an oil under vacuum; yield: 0.65 g (99 %) which was used without further purification; $\nu_{\max}/\text{cm}^{-1}$ (film): 2931, 2857 (CH_2 , asym., sym.), 2275 (NCO); $^1\text{H-NMR}$, d_H : 1.03 (9H, s, $t\text{-Bu}$), 4.76 (2H, s, CH_2), 7.23 (2H, d, $J = 8.38$,
 15 $\text{H}_{\text{arom}3+5}$), 7.35 (2H, d, $\text{H}_{\text{arom}2+6}$), 7.37-7.48 (5H, m, Ph), 7.62-7.71 (5H, m, Ph); MS, (EI), (387.55); m/z : 330 ($\text{M} - t\text{-Bu}$, 52), 286 ($\text{M} - t\text{-Bu}$, $\text{M} - t\text{-Bu} - \text{NCO}$, 48), 199 (Ph_2SiOH^+ , 100); $\text{C}_{24}\text{H}_{25}\text{NO}_2\text{Si}$.

$\text{N}^1(4\text{-tert-butyl-di-phenyl-silyl-O-benzyl})\text{N}^3(\text{di-t-butyl-glutamyl})$ urea (18)

20 Method A: To a solution of di-tert-butyl-glutamate hydrochloride (0.46 g, 1.55 mmol) in THF (7 ml) was added triethylamine (0.31 g 3.10 mmol). The isocyanate, 15, (0.60 g, 1.55 mmol) in dry THF (3 ml) was added to the glutamate ester at room temperature. After 2 h the reaction mixture was filtered and evaporated to
 25 dryness under vacuum. The product was purified by column chromatography (EtOAc : cyclohexane 2:1) resulting in the oil, 18; yield 0.53 g (53%). $\nu_{\max}/\text{cm}^{-1}$ (film): 3359 (NH), 2932, 2857 (CH_2 , asym., sym.), 1729 (C=O , ester), 1670 (C=O , urea), 1154 (C-O , str.); $^1\text{H-NMR}$, d_H : 1.03 (9H, s, $t\text{-Bu}$), 1.40 (9H, s, $t\text{-Bu-glu}$),
 30 1.43 (9H, s, $t\text{-Bu-glu}$), 1.68-2.00 (2H, 2m, $\text{CH}(\text{NH})\text{CH}_2$), 2.18-2.32 (2H, 2m, $\text{CH}_2\text{CO}_2-t\text{-Bu}$), 4.08-4.12 (1H, m, $\text{CH}(\text{NH})\text{CH}_2$), 4.68 (2H, s, CH_2), 6.38 (1H, d, $J = 8.12$, NH-glu), 7.19 (2H, d, $J = 8.41$, $\text{H}_{\text{arom}3+5}$), 7.32-7.47 (7H, m, $\text{Ph} + \text{H}_{\text{arom}2+6}$), 7.62-7.70 (5H, m, Ph), 8.54

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(1H, s, NH-Ph); MS, (EI), (646.90); m/z: 540 (M - t-Bu + 1, 2), 534 (M - 2t-Bu + 2, 5), 478 (M - 3t-Bu + 3, 100), 199 (Ph₂SiOH⁺, 100); C₃₇H₅₀N₂O₆Si.

Method B: (one pot synthesis of compound 18) To a solution of
 5 di-tert-butyl-glutamate hydrochloride (4.14 g, 14.0 mmol) and
 triphosgene (1.39 g, 4.67 mmol) in toluene at -78 °C,
 triethylamine (2.83 g, 28.0 mmol) in toluene (10 ml) was added
 dropwise over 30 min. The reaction was allowed to warm to room
 temperature. After 50 min, a solution containing (4-amino-
 10 benzyl)tert-butyl-diphenyl-silyl ether, 14 (5.00 g, 13.8 mmol)
 and triethylamine (1.95 ml, 14.0 mmol) was added over 5-10 min.
 After 20 h, the reaction mixture was filtered, washed
 sequentially with: H₂O (200 ml), aq HCl (1%, 200 ml), aq Na₂CO₃
 (1%, 200 ml), H₂O (2 x 200 ml) dried (Mg₂SO₄) and evaporated to
 15 an oil under vacuum; yield: 9.90 g. This product was deprotected
 without further purification.

N¹(4-hydroxybenzyl)N³(di-tert-butyl-glutamyl) urea (19)

From Method A: To a solution of 18, (0.53 g, 0.80 mmol) in THF
 (10 ml) was added tetra-butylammonium fluoride (2.5 ml, 2.5 mmol
 20 of 1M solution) in THF at room temperature. After 3 h, the
 reaction mixture was evaporated to dryness under vacuum. The
 product was dissolved in EtOAc (20ml), washed with H₂O (2 x 10
 ml), dried (MgSO₄) and evaporated to an oil; yield: 0.40 g.

The deprotected compound, 19 (0.38 g), was purified by column
 25 chromatography (EtOAc : cyclohexane 3:1) resulting in an oil
 which crystallised on standing; yield 0.093 g (29%). $\nu_{\max}/\text{cm}^{-1}$
 (film): 3370 (broad, NH+OH), 2967 (CH₃), 2930, 2857 (CH₂, asym.,
 sym.), 1716 (C=O, ester), 1678 (C=O, urea), 1153 (C-O, str.); ¹H-
 NMR, δ_{H} : 1.40 (9H, s, t-Bu), 1.42 (9H, s, t-Bu), 1.72-2.00 (2H,
 30 2m, CH(NH)CH₂), 2.20-2.31 (2H, 2m, CH₂CO₂-t-Bu), 4.10-4.18 (1H,
 m, CH(NH)CH₂), 4.39 (2H, d, J = 5.36, CH₂), 4.99 (1H, t, CH₂OH),
 6.38 (1H, d, J = 8.11, NH-glu), 7.16 (2H, d, J = 8.35, H_{arom3+5}),
 7.31 (2H, d, H_{arom2+6}), 8.50 (1H, s, NH-Ph); MS, (EI), (408.94);
 m/z: 408 (M⁺, 10), 352 (M - t-Bu + 1, 4), 296 (M - 2t-Bu + 2,
 35 14); C₂₁H₃₂N₂O₆.

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From Method B: The one pot procedure yielded 18, which was purified by column chromatography; yield 2.57 g (46% over three steps) which was recrystallised from aq MeOH (60%).

N¹(4'-succinimidyl-oxy-carbonyl-oxy-benzyl)N³(di-tert-butyl-glutamyl)urea (20).

To a solution of N¹(4'-hydroxy-benzyl)N³(di-tert-butyl-glutamyl)urea, 19, (0.200 g, 0.49 mmol) and di-succinimidyl carbonate (0.128 g, 0.50 mmol) in 5.0 ml acetonitrile, 0.041 ml pyridine was added at once at room temperature. After 20 h the reaction mixture was evaporated to dryness under vacuum, the oily residue dissolved in 3.0 ml EtOAc, washed with H₂O (3x3.0 ml), dried (MgSO₄) and evaporated again. 0.151g of a semi-solid resulted, which was purified by column chromatography (eluent: EtOAc: cyclohexane 3:1). Finally 0.088 g (yield: 39%) of a solid resulted.

V_{\max}/cm^{-1} (film): 3369 (NH), 1783 (C=O, carbonate), 1726 (C=O, ester, ketone), 1660 (C=O, urea), 1207, 1156 (C-O, str.); ¹H-NMR, δ_{H} : 1.39 (9H, s, t-Bu), 1.42 (9H, s, t-Bu), 1.80-2.00 (2H, 2m, CH(NH)CH₂), 2.22-2.35 (2H, 2m, CH₂CO₂-t-Bu), 2.60 (4H, s, CH₂-succinimidyl), 4.10-4.20 (1H, m, CH(NH)CH₂), 4.89 (2H, s, CH₂), 6.47 (1H, d, J = 8.10, NH-glu), 7.32 (2H, d, J = 8.57, H_{arom3+5}), 7.40 (2H, d, H_{arom2+6}); MS, (549.58); m/z: 506 (M⁺-CO₂+1, 50), 450 (M⁺-CO₂-C₄H₈+1, 18), 394 (M⁺-CO₂-2C₄H₈+1, 60), 279 (M⁺-CO₂-2C₄H₈, -hydroxysuccinimide +1, 90); C₂₆H₃₅N₃O₁₀.

N¹[-(2(R)-[[2(4'-benzyloxy-carbonyl)-amino-3-mercaptopropyl]-amino]-3-methyl-pentyl]isoleucylhomoserine Lactone] N³(di-tert-butyl-glutamyl) urea (21)

To a solution of 9 in CH₂Cl₂ is added N¹(4-succinimidyl-oxy-carbonyl-oxybenzyl)N³(di-t-butyl-glutamyl) urea, 20 in dry THF and triethylamine at room temperature under N₂. The reaction mixture is evaporated, washed, dried (MgSO₄) and re-evaporated to 21.

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N¹[(4-benzylidene-malononitrile-oxy-carbonyl)-4-oxy
benzyl)]N³glutamyl urea (22)

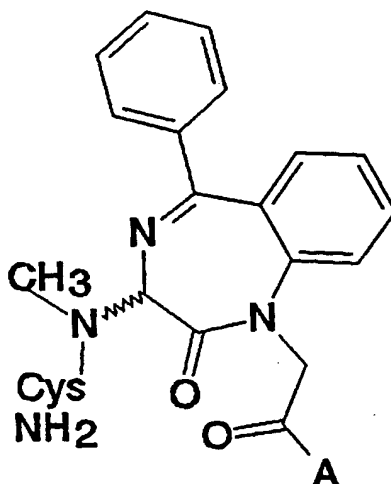
Compound, 21 is dissolved in formic acid (95%), at 4 °C under N₂.
After 22 h the solvent is evaporated under vacuum (pump) to give

5 22.

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CLAIMS

1. A compound which comprises a prodrug of a ras inhibitor (FTLi) linked to at least one protecting group said group being capable of being cleaved from said compound to release the ras inhibitor or a physiologically acceptable derivative of said prodrug.
2. A compound according to claim 1 wherein the FTLi is a farnesyltransferase inhibitor.
3. A compound according to claim 1 or 2 wherein the FTLi is of the formula (Ia)



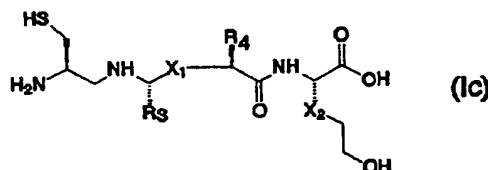
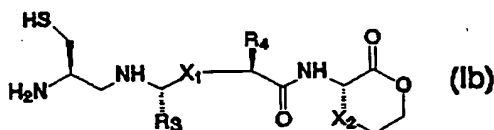
wherein Cys is cysteine, A is a group $-\text{NH CH}(\text{R}^1) \cdot \text{COOR}^2$ wherein R^1 is a naturally occurring amino acid side chain and R^2 is hydrogen, methyl or amino.

4. A compound according to claim 3 wherein A is:
 - NH.CH(CH₂CH(CH₃)₂).COOH;
 - NH.CH(CH₂OH).COOH;
 - NH.CH(CH₂CH₂SCCH₃).COOH; or

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-NH.CH(CH₂CH₂SCH₃) CONH₂.

5. A compound according to claim 1 or 2 wherein the FTLi is of the formula (Ib) or (Ic):

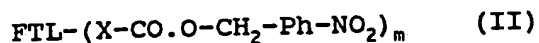


- wherein X₁ is .CH₂CH₂, trans CH=CH or CH₂NH; R₃ and R₄ are the same or different and are the side chains of naturally occurring amino acids, including their oxidized forms, or substituted or unsubstituted aliphatic, aromatic or heteroaromatic groups, and X₂ is (CH₂)_n wherein n is 0, 1 or 2.
6. A compound according to claim 5 wherein R₃ and R₄ are the same or different and are cyclohexyl, phenyl, pyridyl, imidazolyl or saturated or unsaturated straight or branched chains of 2 to 8 carbon atoms optionally substituted with an aromatic or heteroaromatic ring.
7. A compound according to claim 5 or 6 wherein R₃ and R₄ independently represent -CH₃, -CH(CH₃)₂, -CH₂CH(CH₃)₂, -CH(CH₃)CH₂CH₃, -CH₂OH, -CH(OH)CH₃, -CH₂CH₂SCH₃, -CH₂CH₂S(O)CH₃, -CH₂CH₂S(O)₂CH₃.
8. A compound according to claim 5, 6 or 7 wherein R₃ and R₄ are

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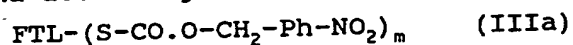
each $-\text{CH}(\text{CH}_3)\text{CH}_2\text{CH}_3$.

9. A compound according to any one of claims 1 to 8 of the formula (II):



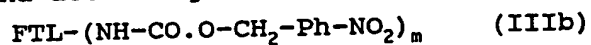
where X which may be the same or different is NH, O or S, m is an integer from 1 to 5, Ph which when m is from 2 to 5 may be the same or different is an optionally substituted phenylene ring and FTL is a group such that $\text{FTL}-(\text{XH})_m$ is a FTLi containing m -XH groups, and wherein the nitro group is in the 2- or 4-position of the Ph ring.

10. A compound according to claim 9 of the formula (IIIa):



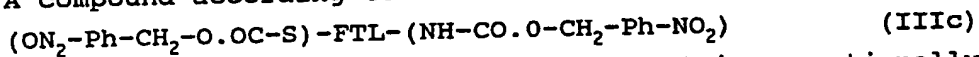
where m is an integer from 1 to 5, Ph which when m is from 2 to 5 may be the same or different is an optionally substituted phenylene ring and FTL is a group such that $\text{FTL}-(\text{SH})_m$ is a FTLi compound containing m mercapto groups, and wherein the nitro group is in the 2- or 4-position of the Ph ring.

11. A compound according to claim 9 of the formula (IIIb):



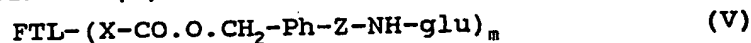
where m is an integer from 1 to 5, Ph which when m is from 2 to 5 may be the same or different is an optionally substituted phenylene ring and FTL is a group such that $\text{FTL}-(\text{NH}_2)_m$ is a FTLi compound containing m amino groups, and wherein the nitro group is in the 2- or 4-position of the Ph ring.

12. A compound according to claim 9 of the formula (IIIc):

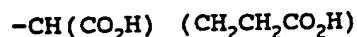


wherein Ph which is the same or different is an optionally substituted phenylene ring and FTL is a group such that $(\text{SH})-\text{FTL}-(\text{NH}_2)$ is a FTLi compound, and wherein the nitro group is in the 2- or 4-position of the Ph ring.

13. A compound according to any one of claims 1 to 8 of the formula (V):

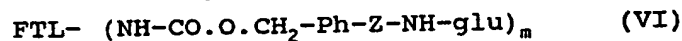


where X, which may be the same or different, is NH, O or S, m is an integer from 1 to 5, FTL is the residue of a FTL compound such that $\text{FTL}-(\text{XH})_m$ is a FTLi compound containing m -XH groups, Ph which when m is from 2 to 5 may be the same or different is an optionally substituted phenyl group, Z is -O.CO- or -NH.CO- and glu is the residue of glutamic acid of formula:

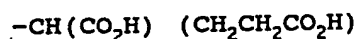


or a di-C₁₋₆ alkyl ester thereof.

14. A compound according to claim 13 of the formula (VI):



where m is an integer from 1 to 5, FTL is the residue of a FTL compound such that $\text{FTL}-(\text{NH}_2)_m$ is a FTLi containing m amino groups, Ph which when m is from 2 to 5 may be the same or different is an optionally substituted phenyl group, Z is -O.CO- or -NH.CO- and glu is the residue of glutamic acid of the formula:



or a di-C₁₋₆ alkyl ester thereof.

15. A compound according to any one of claims 9 to 14 wherein the phenylene ring (Ph) is substituted with 1 to 4 groups which may be the same or different which are selected from fluorine, chlorine, bromine, iodine, hydroxy, mercapto, amino, nitro, C₁₋₄ alkyl, C₁₋₄ haloalkyl, C₁₋₄ alkoxy, C₁₋₄ haloalkoxy, C₂₋₄ alkenyl, C₂₋₄ alkynyl, and C₂₋₄ haloalkenyl.

16. A compound according to claim 15 wherein the ring is substituted at one or more of the 2-, 3-, 5- and/or 6-positions with fluorine.

17. A composition comprising a compound according to any one

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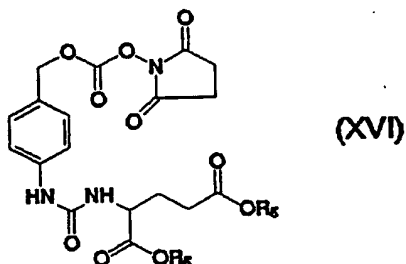
of claims 1 to 16 together with one or more pharmaceutically acceptable carriers or diluents.

18. A system for use in the control of neoplasia in a human or animal subject comprising:
 - (i) an enzyme capable of converting a compound according to any one of claims 1 to 16 to an active drug conjugated with a targeting agent capable of binding to a tumour-associated antigen; and
 - (ii) a compound according to any one of claims 1 to 16.
19. A system for use in the control of neoplasia in a human or animal subject comprising
 - (i) a modified virus capable of selectively infecting tumour cells in said subject, said virus carrying a DNA or RNA sequence encoding an enzyme, and
 - (ii) a compound according to any one of claims 1 to 16 capable of being converted to an active drug by the action of said enzyme.
20. A system for use in the control of neoplasia in a human or animal subject comprising
 - (i) a non viral delivery system capable of selectively infecting tumour cells in said subject, said delivery system carrying a DNA or RNA sequence encoding an enzyme, and
 - (ii) a compound according to any one of claims 1 to 16 capable of being converted to an active drug by the action of said enzyme.
21. A system according to claim 18, 19 or 20 wherein the enzyme is a bacterial nitroreductase or a carboxypeptidase.
22. A process for the production of a compound of the formula $\text{FTLi}-(\text{PRT})_m$ where FTLi is a compound of formula (Ia), (Ib), or (Ic) as defined in claims 3 and 5 provided that each said compound comprises at least one mercapto, hydroxy or

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amino group; m is an integer from 1 to 5; and PRT which may be the same or different when m is from 2 to 5 is a protecting group capable of being cleaved from the compound of formula (Ia), (Ib) or (Ic) by the action of an enzyme; wherein the compound of formula (Ia), (Ib) or (Ic) is linked to the group PRT via said at least one mercapto, hydroxy or amino group; said process comprising: reacting said protecting groups or precursor thereof with a compound of formula (Ia), (Ib) or (Ic) under conditions which produce a compound of formula $\text{FTLi}-(\text{PRT})_m$.

23. A process according to claim 22 wherein the compound produced is as defined in any one of claims 1 to 16.
24. A compound of formula (XVI):



wherein R^5 is C_{1-6} alkyl.

25. A compound according to claim 24 wherein R^5 is ethyl or t-butyl.
26. A process according to claim 22 wherein said precursor is a compound of formula (XVI) as defined in claim 24 or 25, and wherein said process further comprises the step of removing the alkyl groups of the precursor.

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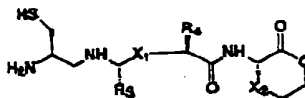
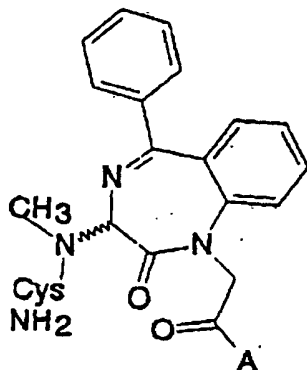
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International Bureau



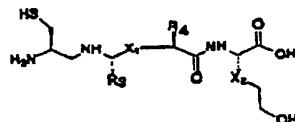
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(71)(72) Applicants and Inventors: SPRINGER, Caroline, Joy [GB/GB]; The Institute of Cancer Research, The Royal Marsden Hospital, 15 Cotswold Road, Belmont, Sutton, Surrey SM2 2NG (GB). MARAIS, Richard [GB/GB]; Chester Beatty Laboratories, The Institute of Cancer Research, 237 Fulham Road, London SW3 6JB (GB).		(88) Date of publication of the international search report: 6 April 1995 (06.04.95)	
(74) Agents: BRASNETT, Adrian, Hugh et al.; J.A. Kemp & Co., 14 South Square, Gray's Inn, London WC1R 5LX (GB).			

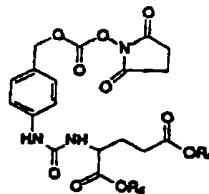
(54) Title: PRODRUGS OF FARNESYL TRANSFERASE INHIBITORS FOR ADEPT OR VDEPT



(Ib)



(Ic)



(57) Abstract

The present invention provides a prodrug of the general formula FTLi-(PRT)_m, where FTLi is a ras inhibitor such as a farnesyltransferase inhibitor compound and PRT represents m protecting groups capable of being cleaved from the ras inhibitor by the action of an enzyme, where m is an integer from 1 to 5.

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INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K47/48 A61K31/55 A61K31/40 A61K31/365

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K C07C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,Y	SCIENCE, VOL. 260, NO. 5116, PAGE(S) 1934-7, 25 June 1993 Kohl, NE. et al 'Selective inhibition of ras-dependent transformation by a farnesyltransferase inhibitor.' see abstract see page 1936	1-4, 9-12, 15, 17-23
X,Y	FASEB J, VOL. 7, NO. 7, PAGE(S) A1048, ABSTR. NO. S60, 20-04-1993 Gibbs JB et al 'Selective inhibition of Ras-dependent cell transformation by a farnesyl -protein transferase inhibitor (Meeting abstract).' see abstract	1-4, 9-12, 15, 17-23

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☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *G* document member of the same patent family

Date of the actual completion of the international search

24 February 1995

Date of mailing of the international search report

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,Y	EP,A,0 547 669 (MERCK & CO. INC.) 23 June 1993 see claims 1,2; examples ---	1-4, 9-12,15, 17-23
X,Y	EP,A,0 547 671 (MERCK & CO. INC.) 23 June 1993 see claim 1; examples ---	1-4, 9-12,15, 17-23
X,Y	EP,A,0 547 672 (MERCK & CO. INC.) 23 June 1993 see claims 1,2; examples ---	1-4, 9-12,15, 17-23
X,Y	SCIENCE, US, VOL. 260, NO. 5116, PAGE(S) 1937-42, 25 June 1993 James GL et al 'Benzodiazepine peptidomimetics: potent inhibitors of Ras farnesylation in animal cells' see figures; tables see page 1941, right column ---	1-4, 9-12,15, 17-23
X,Y	SCIENCE, VOL. 260, NO. 5116, PAGE(S) 1877-8, 25 June 1993 Travis J 'Novel anticancer agents move closer to reality' see the whole document ---	1-4, 9-12,15, 17-23
Y	J. ORG. CHEM.;1992; VOL.57, NO. 6; PAGE(S) 1702-6 Getz J J et al 'Mechanism of hydrolysis of benzamidomethyl derivatives of phenols and its implications for prodrug design' see figures; tables see schemes ---	1-4, 9-12,15, 17-23
Y	J. CHEM. SOC., PERKIN TRANS. 2; 1992; NO. 7; PAGE(S) 1145-50 Mitchell A G et al 'Prodrugs of phosphonoformate: the effect of para-substituents on the products, kinetics and mechanism of hydrolysis of dibenzyl methoxycarbonylphosphonate' see paragraph "Results and Discussion" ---	1-4, 9-12,15, 17-23
X,Y	EP,A,0 540 263 (CANCER RESEARCH CAMPAIGN TECHNOLOGY LIMITED) 5 May 1993 see page 5, line 50 - page 6, line 25 see examples see claims 1-10 ----- -/--	1-4, 9-12,15, 17-23

INTERNATIONAL SEARCH REPORT

Intern. al Application No
PCT/GB 94/01610

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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X	EP,A,0 535 731 (MERCK & CO INC) 7 April 1993 see claims; examples ---	5-12, 15-23
X	83RD ANNUAL MEETING OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH, SAN DIEGO, CALIFORNIA, USA, MAY 20-23, 1992. SEBTI S M et al 'GROWTH INHIBITION OF RAS ONCOGENE TRANSFORMED CELLS BY RAS CAAX CARBOXY-TERMINAL TETRAPEPTIDES' & PROC AM ASSOC CANCER RES ANNU MEET, VOL. 33, PAGE(S) 371, 1992. see abstract number 2217 ---	5-12, 15-23
P,X	JOURNAL OF BIOLOGICAL CHEMISTRY,, VOL. 268, NO. 28, PAGE(S) 20695-20698, 5 October 1993 Nigam M et al 'Potent inhibition of human tumor p21-ras farnesyltransferase by A-1A-2-lacking p21-ras CA-1A-2X peptidomimetics' see figure 1 ---	5-12, 15-23
P,X	J. BIOL. CHEM., US, VOL. 269, NO. 17, PAGE(S) 12410-12413, 29 April 1994 Qian Y. et al 'Design and structural requirements of potent peptidomimetic inhibitors of p21(ras) farnesyltransferase' see abstract; figures ---	5-12, 15-23
P,X	J. BIOL. CHEM., US, VOL. 268, NO. 25, PAGE(S) 18415-18418,, 5 September 1993 Garcia A.M. et al 'Peptidomimetic inhibitors of ras farnesylation and function in whole cells' see abstract; figures; table 1 ---	5-12, 15-23
P,X	CELL, US, VOL. 77, NO. 2, PAGE(S) 175-178, 22 April 1994 Gibbs J.B. et al 'Farnesyltransferase inhibitors: Ras research yields a potential cancer therapeutic' see the whole document ---	5-12, 15-23
P,X	J MED CHEM, US, VOL. 37, NO. 6, PAGE(S) 725-32, 18 March 1994 Graham SL et al 'Pseudopeptide inhibitors of Ras farnesyl-protein transferase.' see tables --- -/--	5-12, 15-23

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Internal Application No
PCT/GB 94/01610

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
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X	PROC. NATL. ACAD. SCI. U.S.A., 1991, VOL. 88, PAGE(S) 8039-8043 B.E. HUBET ET AL. 'RETROVIRAL-MEDIATED GENE THERAPY FOR THE TREATMENT OF HEPATOCELLULAR CARCINOMA: AN INNOVATIVE APPROACH FOR CANCER THERAPY' cited in the application see the whole document ---	1-12, 15-23
X	FASEB J., US, 1990, VOL. 4, NO. 2, PAGE(S) 188-193 Senter P.D. 'Activation of prodrugs by antibody-enzyme conjugates: A new approach to cancer therapy' see abstract see page 190 see figures ---	1-12, 15-23
Y	J. MED. CHEM., 1990, VOL. 33, PAGE(S) 677-81 Springer, Caroline J. et al 'Novel prodrugs which are activated to cytotoxic alkylating agents by carboxypeptidase G2' see Scheme I see figures; table 1 see Experimental Section ---	5-8, 13-23
P,Y	PROC ANNU MEET AM ASSOC CANCER RES, VOL. 35, PAGE 379, ABSTR. NO. 2259, 1994 Springer CJ et al 'Novel prodrugs for antibody-directed enzyme prodrug therapy (ADEPT) for use in cancer treatment (Meeting abstract).' see abstract ---	5-8, 13-23

INTERNATIONAL SEARCH REPORT

Intern al Application No
PCT/GB 94/01610

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,Y	85TH ANNUAL MEETING OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH, SAN FRANCISCO, CALIFORNIA, USA, APRIL 10-13; 1994, VOL. 35, PAGE(S) 507, ABSTR. NO. 3023. Blakey D C et al 'Anti-tumour effects of an antibody-carboxypeptidase G2 conjugate in combination with a phenol mustard prodrug' see abstract ---	5-8, 13-23
X	EP,A,0 540 263 (CANCER RESEARCH CAMPAIGN TECHNOLOGY LTD.) 5 May 1993 see page 3 see claims; examples ---	1-12, 15-23
P,X	WO,A,94 02450 (ZENECA LTD & CANCER RESEARCH CAMPAIGN TECHNOLOGY LTD.) 3 February 1994 see the whole document ---	5-8, 13-23
T	J. MED. CHEM., VOL. 37, NO. 21, PAGE(S) 3452-8, 14 October 1994 Mauger, Anthony B. et al 'Self-Immolative Prodrugs: Candidates for Antibody-Directed Enzyme Prodrug Therapy in Conjunction with a Nitroreductase Enzyme' see the whole document -----	1-12, 15-23

INTERNATIONAL SEARCH REPORT

International application No.

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Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☒ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
PLEASE SEE ATTACHED SHEET ../..
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

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1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
1. claims 1-4, 9-12, 15-23
2. claims 5-12, 13-23
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☒ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/GB94/01610

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

LACK OF UNITY OF INVENTION

- 1 YES Claims 1-4, 9-12, and 15-23; relating to prodrugs of a benzodiazepine structure, derivatised with a $\text{CO.O-CH}_2\text{-Ph-NO}_2$ radical.
- 2 NO Claims 1-4, 13-23; relating to a prodrug of a benzodiazepine structure, derivatised with a $\text{CO.O-CH}_2\text{-Ph-Z-NH-glu}$ radical.
- 3 NO Claims 5-12 and 13-23; relating to a prodrug of a peptide-like structure.
- 4 NO claims 15-23 in part, and 24-26; relating to a prodrug of a structure as described in claim 24.

The problem underlying the present application is, in its broadest form, the provision of new prodrugs, that can be used in the treatment of certain types of cancer, acting through the inhibition of ras, or more precisely, through the inhibition of farnesyl transferase.

As solution to this problem, two types of inhibitors of farnesyl transferase are used: see claims 3 and 5.

The possibilities of making a prodrug from these compounds are given in claims 9-14: using either a $\text{CO.O-CH}_2\text{-Ph-NO}_2$ (claims 9-12) or a $\text{CO.O-CH}_2\text{-Ph-Z-NH-glu}$ (claims 13-14) radical.

Moreover, a separate group of compounds is given in claim 24. In example 2, one of these compounds is indicated as "a novel ras prodrug cleavable by the enzyme CPG2". It is, however, not clear which part of the molecule of the actual inhibitor.

The special technical feature, linking these solutions together, is the use of a prodrug of a farnesyl transferase inhibitor in cancer therapy.

The use of inhibitors of farnesyl transferase and their prodrugs in cancer therapy is known. The mechanism for this use is explained in Science, pages 1877-1878. The use of a prodrug on this inhibition is known from Science, pages 1934-1937 (see the abstract) and FASEB J. (see the abstract cited).

For this reason, the special technical feature mentioned above can no longer be accepted as technical feature linking the different inventions together.

In principle, the structure of the inhibitor could serve as special technical feature, linking the different prodrugs together. However, in the first group of inhibitors, i.e. the benzodiazepines, several derivatisations are known from Science, pages 1937-1942. Therefore, this subject further lacks unity of invention.

Since searching this plurality of different subjects would have caused major additional searching efforts, a search was performed for the first subject only.

It is to be noted, that each of the subjects not searched may, after the retrieval of new documents, give rise to further objections for lack of unity.

INTERNATIONAL SEARCH REPORT

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FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

OBSCURITIES, ETC.

In view of the large number of compounds, which are defined by the general definitions of the compounds to be used, the search had to be restricted for economic reasons. The search was limited to the compounds for which pharmacological data was given and/or the compounds mentioned in the claims, and to the general idea underlying the application (see guidelines, Part B, Chapter III, paragraph 3.6).

Partially searched claims: 1-2, 9-12, 15-23
Partially searchable claims: 1-2, 5-23

INTERNATIONAL SEARCH REPORT

Information on patent family members

Intern. Application No

PCT/GB 94/01610

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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INTERNATIONAL SEARCH REPORT

Information on patent family members

Intern. Application No

PCT/GB 94/01610

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9402450		ZW-A- 9293	30-03-94